Miniprep Plasmid Extraction

Materials and Equipment

- Conical tubes (15 ml)
- Alcohol lamp
- Microtube rack
- Lighter
- Micropipette
- Micropipette tips (blue and yellow)
- Microtubes (2 ml)
- Shaker
- Water bath

Reagents

- Liquid LB medium with proper antibiotic (KAN 15 mg/ml, CAM 35 mg/ml, AMP 100 mg/ml)
- STE buffer
- Lysozyme (10 mg/ml)
- RNase A (200 ug/ml)
- Sodium Acetate (3M, pH 5.2)
- Isopropanol
- Ethanol 70%
- Nuclease free water

Methodology

1. Culture 1 ml of transformed bacterial strain into a 50 ml flask containing LB with the proper antibiotic.
2. Incubate ON, 37˚C, 250 rpm.
3. Place the flask on ice for 20 minutes.
4. Centrifuge 13,000 rpm for 2 minutes.
5. Discard supernatant.
6. Resuspend with 350 µl of STE buffer and start timer.
7. Transfer to a 2 ml microtube.
8. Add 3 µl of lysozyme per tube.
9. Incubate at room temperature until timer marks 4 minutes.
10. Place microtubes in a boiling water bath for 1 minute to inactivate the lysozyme, then transfer microtubes to ice for 2 minutes.
11. Centrifuge at 13,000 rpm for 10 minutes.
12. Discard pellet and add 4µl of RNAse A (200 ug/ml) to the liquid phase.
13. Incubate at room temperature for 15 minutes.
14. Add 75 µl of sodium acetate and 400 µl of isopropanol.
15. Stir gently and incubate for 10 minutes at room temperature.
16. Centrifuge at 13,500 rpm for 10 minutes and discard supernatant.
17. Wash pellet twice by resuspension with 70% ethanol.
18. Let the microtube dry for 45 minutes. OPTIONAL: Place microtubes on thermoblock for 15 min at 65˚C, for a faster drying.
19. Resuspend in 100 µl molecular biology grade water.